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(71) Applicant *(for all designated States except US)*: OY MEDIX BIOCHEMICA AB [FI/FI]; Asematie 13, FIN-02700 Kauniainen (FI).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: KOUVONEN, Ilkka, Sakari [FI/FI]; Kandidaatintie 10 B, FIN-02700 Kauniainen (FI).

(74) Agent: BORENIUS & CO. OY AB; Kansakoulukuja 3, FIN-00100 Helsinki (FI).

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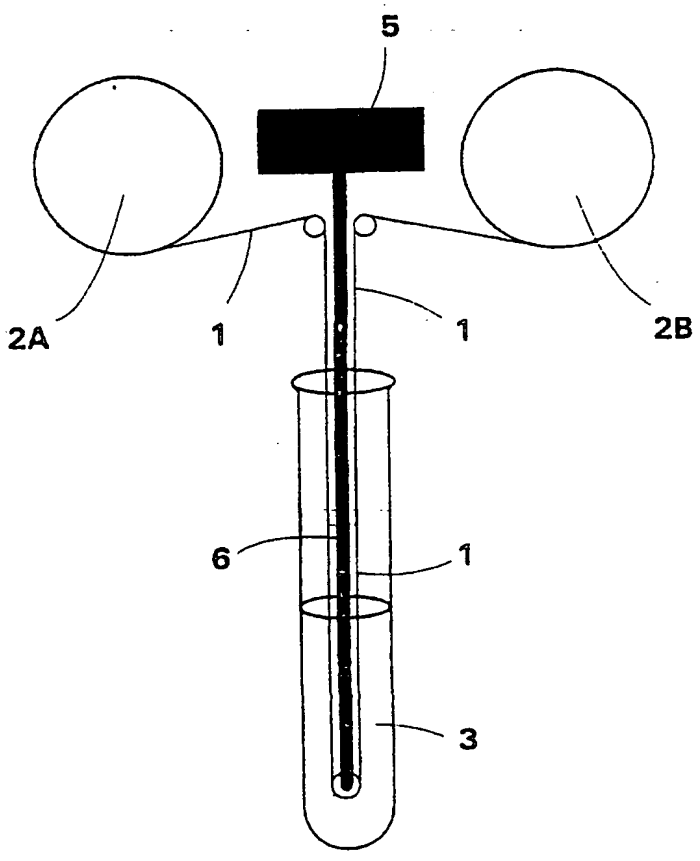
(54) Title: COATED FLEXIBLE THREAD-LIKE SOLID CARRIER FOR IMMUNOASSAYS

(57) Abstract

The present invention is related to a flexible thread-like solid carrier coated with a specific binding substance, e.g. antibodies. The thread-like solid carrier can be used in immunoassays and is spooled around a coil, which can be mounted on a support, which can be installed, e.g. in an automatic analysator, which allows the thread-like solid carrier to contact the sample, the appropriate substrates and recording systems before it is reeled up on another mountable coil.



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COATED FLEXIBLE THREAD-LIKE SOLID CARRIER FOR IMMUNOASSAYS

The Technical Field

The present invention is related to a flexible thread-like solid carrier coated with a specific binding substance, a device such as a coil on which said flexible thread-like solid carrier is spooled as well as the use of said flexible thread-like coated solid carrier and at least one coil carrying said solid carrier in immunoassays suited for automation and continuous measurement. The invention is also related to immunochemical methods in which said solid carrier and coil(s) are used.

The Background of the Invention

Immunochemical methods frequently use solid phase techniques, in which the specific binding substances, such as antibodies, receptors, synthetic binders, single stranded nucleic acid sequences etc., are attached to a solid surface. The separation of bound and unbound specific binding substances, such as antibodies, required in non-homogenous immunoassays, is easily achieved by washing and removing said solid surface. One of the oldest applications of the technique is the immunoradiometric assays, IRMA.

The basic principle of the techniques in one of its simplest forms is to coat the inner surface of a test tube with an excess of a specific binding substance, such as an antibody. The test sample is added to the coated tube and during the incubation the analyte such as an antigen in the sample is immobilized by binding to the antibody on the surface of the test tube. The unbound sample is removed after a suitable incubation time. The test tube is washed or rinsed at least once by adding one or several lots of a buffer solution to

remove the surplus of unbound analyte as well as other components in the sample. Said components must be removed because they might disturb the determination. Thereafter, a solution containing a label is added. For example, in the IRMA a radiolabelled antibody distinguishable from the immobilized antibody is used. The labelled antibody is added into the empty washed test tube.

The analyte or antigen from the sample, which has been captured by the immobilized antibody on the surface of the test tube binds an amount of the labelled antibody, which correlates with the amount of analyte in the sample. The test tube is washed or rinsed again to remove the surplus of the labelled antibody. The amount of bound label is measured using different techniques.

Many different modifications of the solid phase technique exist. Instead of coating the inner surface of test tubes, the inner surface of the wells of microtiter plates can be coated. Alternatively, the specific binding substance is attached to the surface of glass or plastic beads, which are easy to separate, wash and remove. Some test-kits for immunoassays are developed based on the idea that the specific binding substance is attached to the surface of a plastic sheet. Said sheet is immersed or dipped into the sample and thereafter into different solutions containing buffers for washing or rinsing and into mixtures with reagents, such as labelled substances to achieve a detectable reaction. In immunochromatographic methods a membrane of nitrocellulose is coated. The sample is allowed to migrate through said nitrocellulose membrane, which binds from the sample the labelled molecule-complexes, which are to be determined.

The latest developments in solid phase techniques are related to immunosensors, which are very promising for quantitative analysis. One interesting approach is the development of

optical immunosensors. The solid phase consists of optical fibers, which are coated with binding substances such as enzymes. Small changes caused by binding reactions are recorded with suitable detectors. These new systems are discussed in detail for example by Morgan, C.L., et al. in Clinical Chemistry 42:2, 193-209, 1996.

Frequently used solid phase techniques are for example a test developed for diagnosing different types of allergy, in which test antigens attached to pieces of cellulose are added into the sample. Most modern automatic devices for immunochemical determinations apply magnetic particles coated with specific binding substances. After the reaction, the particles are separated from the solution by the aid of magnets. Usually, disposable cuvettes or casettes containing the required amount of particles are used.

Typical previously used solid carriers are test tubes, beads, sheets, but also the use of cloth made of polypropylene, polyester, nylon and polyethylene has been suggested (US 5,169,757,; US 5,424,220; and US 5,122,452). However, it has not previously been suggested that such cloth-like bands could be spooled or reeled on a coil and be transferred or moved from one coil to another.

A multiple binding assay system with a plurality of coated filaments or threads attached to an elongated support for use in allergy test is described in US 4,459,360.

EP 451 686 discloses a flow-through immunoassay test device in which a control reagent is incorporated with a thread element extending across the surface of a test surface.

Even if threads coated with specific binding substances have been used in immunoassays, so far they have not been used as the sole carrier of the immobilized specific binding subs-

tance, but have been attached to or used in combination with other solid supports. A solid carrier coated with a specific binding substance in the form of a thread, which can be transported from one place to another through a chain of reagents is a totally new concept, which has not been used in previous immunoassay applications.

The most pertinent problem connected with the prior art methods is the separation of the antibody-analyte and antibody-analyte-labelled antibody-complexes.

The previous automated solid phase applications involve many steps and consist of complicated mechanical structures. From the production point of view, separated particles and wells of microtiter plates are difficult and complicated to manage during the coating procedure.

The transfer of a flexible thread-like solid carrier can be performed in a simple mechanical manner, but it can also be highly automatized and computerized. Said flexible thread-like solid carrier can be kept in the automatic device and applied precisely in the required amounts.

Assemblies, sets or batteries of coils containing said flexible thread-like solid carriers coated with different specific binding substances can be developed for each analyte to be tested e.g. in a clinical laboratory. These coils can be mounted in different ways in different kinds of automatic equipment, which are capable of transferring the thread through appropriate reagents and recording systems.

In one specific embodiment of this invention the coils are for example mounted on a support provided with a needle-like device, the pin, and said support can be attached to a rotatable arm and thus easily removed from or inserted into an automatic determination apparatus. Thus, the apparatus is easy to apply

in different types of analyses. The amount of waste generated by the solid phase of the present invention is very small. The thread coated with the specific binding substance can be applied in manual systems as well as in highly sophisticated automated systems.

In another specific embodiment of this invention the coils are mounted so that the thread-like solid carrier is continuously running through the sample solution and the appropriate reagents and the results are recorded automatically. For example a change in the recorded results can be made alert an alarm system.

The main objective of the present invention is to provide a simple mechanical immunoassay, which can be highly automated and computerized and allows continuous measurements, e.g. in clinical immunoassays.

Another objective of the present invention is to provide an easy way for automatic control of industrial processes as well as the control of the quality of raw water supplies, waste waters and other effluents. These continuous immunoassay systems allows follow-up studies of the concentration of an analyte continuously and recording on-line.

A further objective is to provide a precise immunoassay, in which the dosage of the specific binding substance is very exact, but still easy to change for other uses. For example, the sensitivity is easy to regulate, by changing for example the length of the thread contacted with the sample.

A further objective is to provide a solid phase, which is easy to store and requires little space and has a good stability during storage. It is easy to prepare a set or battery of different coils containing the flexible thread-like solid carriers coated with different specific binding substances for

various types of tests, e.g the most frequently used clinical immunoassays. The thread-like solid carriers can be kept in the automatic device and applied precisely in the required amounts, which are easy to regulate by changing the length of the thread contacted with the sample.

Another of the objectives of the present invention is to provide an environmental friendly immunoassay system through decreased consumption of reagents, washing solutions and disposable kits.

The Summary of the Invention

The present invention provides a flexible thread-like solid carrier for use in immunoassays the characteristics of which are set out in the claims.

The subject matter of this invention comprises a flexible thread-like solid carrier coated with at least one specific binding substance. The thread-like solid carrier consists of one or more thin and even strands, which can be twined. It should be flexible, i.e. bendable and reelable around a coil, but not too elastic. Preferably it should be prepared of polyester or polypropylene, but also cotton and other materials, e.g. plastics are useful. The thickness of the solid carrier is not an essential prerequisite. It is more important that the solid carrier is flexible and reelable and can be adjusted to the equipment in which it is used.

The flexible thread-like solid carrier can be treated with surface modifying washing solutions such as detergents, ethanol, dioxine or other contaminant removing eluents to enable a better attachment of the specific binding substance during the coating procedure. The thread-like solid carrier is spooled or winded up on the coil, which can be inserted into an automatic or continuously working analytical device or apparatus.

For manual use, the solid carrier or thread is precut in pieces of defined length. Thus, the invention provides a solid phase system, which is easy to handle both manually and automatically.

The invention relates to a method for performing immunoassays for at least one analyte. It is essential for the method that the flexible thread-like solid carrier can be mounted on a coil and the thread can be automatically transferred through the appropriate reagents and recording systems and the spent solid carrier is reeled up on another coil.

In the following some specific non-restrictive embodiments of the invention are described. It is self-evident for those skilled in the art to apply the invention in other methods and equipments.

One specific embodiment of the invention relates to a method for performing immunoassays for at least one analyte, comprising the steps of:

(a) contacting a defined part of the flexible thread-like solid carrier coated with a specific binding substance to allow the analyte of the sample to react with said specific binding substance for a predefined time;
(b) transferring said flexible thread-like solid carrier continuously or step-wise into a cuvette containing a washing solution;
(c) transferring said thread-like solid carrier into a cuvette containing another labelled binding substance specific for the analyte;
(d) transferring said thread-like into a cuvette containing a second washing solution;
(e) recording the results obtained with a direct label or optionally if e.g. an enzymatic label is used transferring said thread into a cuvette containing a substrate, which is allowed to react with the label so that a detectable

signal can be recorded; and

(f) collecting the spent thread-like solid carrier on another coil.

It is to observed that steps (b) - (e) are non-restrictive and applicable to any methods or recording systems available.

The method of the invention is suited for performing immunoassays with a multitude of different analytes, wherein a separate coil comprising a thread coated with a binding substance, which binds to the analyte to be determined is installed for each type of analyte to be determined.

In the following we describe some other nonrestrictive embodiments of the devices according to the present invention.

The invention is e.g. related to a device, which comprises at least one coil for spooling the unused flexible thread-like solid carrier coated with a specific binding substance. The coil or coils with the coated thread-like solid carrier is/are mounted on a support provided with a needle-like device, the pin, which can be attached to a rotating arm in an automatic analysator. The needle-like device is provided with facilities for fastening and transferring the thread-like solid carrier, e.g. with a needle's eye or one or more loops or a forklike systems, which keeps the thread-like solid carrier in the right position during the performance of the automatic equipment. Said pin acts as a support in the transporting and attaching of the thread in a transferable manner to another easily removable coil, which collects or recoils the spent thread.

An assembly, set or battery of devices or coils with threads coated with different types of binding substances makes it easy to change the test from one type to another.

Another non-restrictive embodiment of the invention is designed to be used in an apparatus for continuous measurements well-known in the art, in which the invention comprises the following details:

(a) a flexible thread-like solid carrier coated with a specific binding substance;
(b) a coil for spooling said thread-like solid carrier of step (a);
(c) a supporting device for the coil of step (b);
(d) a sample source, e.g. a container or a cuvette;
(e) a needle-like device, the pin, which immerses the thread-like solid carrier into said sample source of step (d)
(f) at least one container for a washing solution;
(g) at least one container for the labelled reagent;
(h) at least one container for an optional further washing solution;
(i) at least one container for a substrate;
(j) at least one recording system; and
(k) a coil for collecting the spent thread-like solid carrier.

A further embodiment of the invention is related to continuous measurements of e.g. effluents or a raw-water stream. Said invention comprises the following details:

(a) a thread-like solid carrier coated with a specific binding substance;
(b) a coil for spooling said thread-like solid carrier of step (a);
(c) a supporting device for introduction of the thread-like solid carrier into a sample source;
(d) facilities for performing the determination including optional washing solutions, labelled reagents, substrates and recording system; and
(e) a coil on which the spent thread-like solid carrier is winded up.

The invention is also related to the use of the thread-like

solid carrier coated with a specific binding substrate mounted on a supporting device such as a coil in an apparatus for automated or continuously working immunoassays.

The use of the coil containing the thread-like solid carrier coated with a specific binding substance in an apparatus for automatized continuously working recording of the results of the immunoreaction.

The Brief Description of the Drawings

Fig. 1 is a schematical, partial cross-section seen from the end of the rotating arm in the device, which is responsible for immersing the coated thread into the cuvettes while the thread is transported from one coil to another.

Fig. 2 is a schematical picture of the apparatus seen from above showing the rotatable arm, a moving band with its peripheral equipment for transporting samples including a battery of cuvettes for the reagents on a turntable.

Fig. 3 schematically depicts the principle for a continuous immunological measurement.

Fig. 4 schematically depicts another principle for a continuous immunological measurement.

Fig. 5 shows the absorbation curves for ethanol-washed threads.

Fig. 6 shows the absorbation curves for dioxin-washed threads.

The Detailed Description of the Invention

Definitions

In this patent application the terms used have the same meaning as generally used in the fields of immunochemistry, immunology, biochemistry, etc. Some terms are used more extensively and have a meaning which somewhat differs from the general use of the term. Some of these terms are defined below.

The term "solid carrier" means a flexible, bendable thread-like support for a specific binding substance for use in immunoassays.

The term "thread" means a single or multiple, optionally twined strand of polyester, polypropylene, cotton, etc. coated with at least one specifically binding substance. The thread is characterized by a good tensile strength and the multiple strands can be loosely or tightly intertwined. It is most advantageous, if the thread is even and smoothly running, but some fluffiness gives more attachment area for coating. The diameter of the thread can be 0.1-3.0 mm, preferably 0.16-2.6 mm, but can vary within wider ranges depending upon the apparatus used and applications.

The term "specific binding substance" means a substance, which is capable of binding to the analyte, such as an antibody, antigen, receptor molecule, synthetic binder and/or single or double stranded nucleic acid sequences, etc.

The term "analyte" means a substance, which is specifically bound to the above defined "specific binding substance". It is the substance to be determined in the sample. It can be an antigen, such as a hormone, drug etc, if the specific binding

substance is an antigen the "analyte" is an antibody", etc.

The term "coil" means a device, such as a spool or reel, on which the thread-like solid carrier can be winded up, spooled or rolled. The coil can easily be mounted into automatic or continuously working analytical devices or apparatuses. The coils are usually two, one for the unused and another for the spent thread-like solid carrier. The coils can be mounted on a support, which can be installed on an automatically working device or apparatus.

The term "pin" means a needle-like device, i.e. a rigid support around which the thread moves. The device can be attached to a rotating arm and comprises at least one system, which allow the free movement of the thread and at the same time keeps it in the desired position.

The term "needle's eye" means at least one hole or a row of holes on the needle-like device, the pin, which enables the user to regulate the length of the thread, which is to be immersed into the sample. Alternatively, a row of loops might be attached to the needle-like device. Alternatively, a fork-like device can be provided for keeping the thread smoothly running in the right position.

The term "sample" means a liquid sample, which can be a clinical sample, such as blood or serum or a liquid sample from any source. It can e.g. be a sample from an industrial process and it can comprise a stream of raw-water or waste-water or the process water, etc. for recording of possible changes in the material to be analysed.

The term "label" means a marking substances, which can be a radioactive, enzymatic, fluorochromic or luminescent substance, which allows the analyte to be detected, measure and

recorded.

Also dispersed dyes, coloured particles, such as latex particles or colloidal gold as well as silica or carbon sols can be used.

The term "pin" means a needle-like device, i.e. a rigid support around which the thread moves. The device can be attached to a rotating arm and comprises a systems, which allow the free movement of the thread and at the same time keeps it in the desired position.

The term "substrate" means a substance, which when allowed to react with the label produces a detectable or measurable signal or colour, which can be recorded.

The term "automatic analysator" means the device or apparatus, which includes the coated thread-like solid carrier and can be developed from any conventional device or apparatus used for automatized, chemical or enzymatical determinations and for continuous measurements.

The the term "support" means a device, on which the coils including the thread-like solid carrier can be fastened for easy installation or mounting in an automated apparatus.
The term "set of devices" means a battery or assembly of coils containing threads coated with different types of binding substances. Said batteries of coils can be assembled in different combinations either containing the same specific binding substance or different kinds of specific binding substances depending upon the needs of the customers.

General Description of the Invention

The present invention provides a flexible and generally applicable solid carrier, such as a thread-like support for a

specific binding substance and a device, such as a coil for winding up the thread-like solid carrier. The thread-like solid carrier and the coil are applicable in automatic devices or apparatuses similar to those used in conventional, automatic chemical or enzymatic determinations. The apparatus, in which the thread and coil of the present invention is to be applied might contain containers, or cuvettes, which can be automatically driven, systems for automatic dosaging of reagents, as well as photometric or fluorometric systems for automatic recording of the results. Such automatic recording systems and apparatuses for continuous chemical and enzymatic measurements have been described e.g. in patents US 4,684,252 and US 4,313,735.

The systems can be computerized and the results recorded automatically by a computer. The coil, reel, roller or shuttle contains the thread-like solid carrier coated with the specific binding substance, preferably an antibody, which specifically binds the analyte, which is to be determined. The coil is mounted, assembled or fitted on a support, which can be installed e.g. on a rotating arm, which is capable of spooling, rolling, driving, winding or reeling the thread and immersing or dipping a clean unused portion or site of the thread into the cuvette for a suitable incubation time, which allows the appropriate binding reaction to take place. After incubation the sample can be removed with suction and the rinsing solutions required in the next step can be added. The labelled reagent is preferably an enzyme, which is detectable by the aid of its effect on a substrate. Said effect is measurable photometrically or fluorometrically. After the recording the thread can be driven forward for the analysis of the next sample by a method or device, which can be selected from a group comprising a multitude of conventional methods and devices. The transfer of the thread-like solid carrier can be performed in a simple mechanical manner, but it can also be highly automated and computerized. The thread-like solid car-

rier can be installed and kept in the automatic device or apparatus and applied precisely in the required amounts. The amount of waste generated during the assay is very small.

Naturally, the thread-like solid carrier coated with the specific binding substance can be applied in manual systems as well. The thread-like solid carrier is easy to handle, it is easy to cut and the dosage-system is very precise. The thread-like solid carrier has a good stability, including storage stability and it is easy to store and it does not require much place. The sensitivity of the test is easy to change by regulating the length of the thread. In the production stage the thread-like solid carrier is easy to handle and cheap to coat and continuous coating processes can be used. For each analyte to be tested, a separate thread-like solid carrier coated with a specific binding substance of said analyte is required.

Each thread-like solid carrier can be reeled on its own coil and an assembly, battery or set of coils, each containing thread-like solid carriers coated with different specific binding substances can be provided and these coils can easily be removed and inserted into the apparatus in which the immunoassay is performed.

In a continuously working, automatic immunoassay device or apparatus, the thread-like solid carrier coated with the specific binding system at first passes the sample and binds to the specific binding substance preattached to the surface of the thread-like solid carrier the substance or analyte, e.g. the antigen, which is to be determined. The thread-like solid carrier can be moved at a continuous steady rate, or step-wise into or through the sample solution. The thread-like solid carrier is allowed to stay in the sample solution for a strictly defined time. The amount of the analyte, which is bound during said time is proportional to the concentration of the

analyte in the sample. The thread-like solid carrier coated with a specific binding substance immobilizes a proportional amount of the analyte during the incubation time. Thereafter, the thread-like solid carrier is directed or driven into at least one washing solution. After the washing step, the thread is contacted with a labelled substance, e.g. an enzyme labelled antibody solution and allowed to react for a suitable time. Thereafter, the thread-like solid carrier is allowed to pass or is immersed in or contacted with at least one washing or rinsing solution and it is directed or driven into a substrate solution, in which the detectable or measurable enzyme reaction is allowed to take place. The amount or concentration of the measurable, e.g. coloured reaction product is proportional with the concentration of the analyte in the sample of interest.

In said or a similar manner, variations or changes in the concentration of the analyte to be determined in the sample can be recorded. The sample solution can be taken from a certain step in a industrial process or it can be a stream or flow of a raw material with varying concentrations of the analyte to be measured.

In the coating process used for attaching the specific binding substance to the thread-like solid carrier, any methods known from conventional solid phase coating systems can be used (Edouard Kurstak: Enzyme Immunodiagnostics, Academic Press, Inc., Florida 1986, pp 13-37 (Principles of the Design of Enzyme Immunoassays/Solid Phases used in Enzyme Immunoassays).

The choice of method depends upon the material of the surface of the thread-like solid carrier. The most frequently used coating system is passive adsorption. Said method is suitable for most materials. If for example a surface of glass or polystyrene is allowed to stay in contact with a relatively concentrated antibody solution for an incubation time of se-

veral hours, a sufficient amount of said antibody is adsorbed or immobilized on the surface. Immunosurfaces with sufficient stability are obtainable with said passive adsorption process. If the material of the thread-like solid carrier, which is intended to be coated contains reactive groups, chemical methods can be used to provide covalent bindings. The antibody can for example be bound to carboxyl groups of the thread by the aid of a carbodiimide catalyzed reaction.

Many different types of thread-like materials can be applied in the present invention. Good coating qualities is an important criteria in the choice of a suitable thread-like solid carrier. In addition, it is important that the thread-like solid carrier is easy to handle, i.e. it should be smooth and even and have a uniform texture, even if some fluffiness might be advantageous and provides additional coating area. However, the thread-like solid carrier should have a sufficient tensile strength and be capable of smooth running. The thread-like solid carrier should preferably have the largest possible surface area per length unit. It can for example consist of several strands even if it has to be sufficiently thin so that it works effectively and is suitable in automatic systems.

In the following examples the invention is described in more detail. These examples are only illustrative and should not be interpreted as limiting the scope of the invention.

Example 1
The coils with coated thread mounted on the support

One preferred embodiment of the invention is shown in Fig. 1. The transferable unused coated thread (1) coated with a specific binding substance is spooled around a coil (2A) and the used thread is collected on another coil (2B) after first being brought into contact with the sample (3) in e.g a cuvette or test tube and then with other reagents.

The coils (2A, 2B) are attached to a supporting device (5) and the thread (1) moves along a needle-like device, the pin (6), attached to a rotatable arm or shaft (7). The pin (6) is always immersed to an equal depth into the sample solution (3). The depth is automatically controlled or measured from the surface of the sample (3), thus allowing a uniform and controllable length of the thread (1) to be contacted with the sample (3).

Example 2
The coils installed in an automatic apparatus

The principles of the device are also shown in Fig. 2, in which the coil (22A) carrying the coated thread-like solid carrier (21) and moving to the coil (22B) where the spent thread-like solid carrier is assembled by the aid of the supporting device (25), which can be mounted or installed on the rotating arm or shaft (27). Figure 2 also shows the test samples (23), which move continuously or step-wise along a transporting path (24), which can be part of a turntable or rotating path (28). The rotating arm or shaft (27), is attached by at a conventional central unit to the turntable or rotating path (28) and the transporting path (24) containing the reagents (29). The central unit allows a rotating backward and forward movement as well as a up and down directed movement of the rotating arm or shaft into the cuvettes containing the samples (23) and to a variable amount of the reagent supplies (29), containing the appropriate washing solutions, label-containing solutions, substrates etc. Along the transporting path (28) several rotating shafts (27) can be assembled in an appropriate manner suited for the test to be made.

Example 3
Continuous immunological measurements

a) Application, wherein the thread is immersed into the sample

In Fig. 3 the principles of a continuously working immunological test system is shown. The coated thread (31) is spooled on a coil (32A) and moves through the appropriate reagent solution cuvettes (34A, 34B, 34C, 34D, etc.) to the coil (32B) on which the spent thread is respooled. The coated thread (31) first moves into the sample (33), which can be e.g. a raw water supply. It continues through the reagent solution cuvettes (34A, 34B, 34C, etc.) to a flow through cuvette (39), in which the thread (31) is met by a stream of the appropriate substrate (34D) and the coloured substance produced with the substrate is measured before the spent coated thread (31) is rolled up on coil (32B), which is discarded after all the thread is used.

b) Application, wherein the thread is running through the sample

In Fig. 4 the principles of a continuously working immunological test system is shown. The coated thread (41) is spooled on a coil (42A) and moves through the sample, which can be raw-water supply and then through a recording system (44), which means any system based on conventional solid phase immunoassay methods and recording devices available or to be developed in the future. The thread then moves to the coil (42B) on which spent thread (41) is respooled. As a uniform quality is expected any changes in the recorded results causes an alarm.

Example 4
Coating systems

In all examples the same coating system i.e. passive adsorption is used. The threads were coated with anti-hCG 5008 (Oy Medix Biochemica Ab) and they were used as the solid-phase in an enzyme-immunological hCG-determination.

Before coating the threads were washed with ethanol and mixed for one hour by a horizontal shaker. The ethanol was removed by suction and the threads were washed twice with water.

The threads were coated in a coating buffer (10 µg antibody/ml PBS). The coating solution was allowed to act on the thread overnight at room temperature. The coating solution was removed by suction and the thread washed thrice with a buffer (PBS containing 0.05 % Tween 20). Blocking solution (50 mM Na-phosphate, 5 mg/ml BSA, pH 6.5) was added and allowed to act overnight at +4°C and removed by suction.

The threads were allowed to dry at +35°C overnight and kept for storage at a temperature of +4°C. The coated dried threads were packed into bags of aluminium laminate (folio).

Example 5
Types of threads used as solid phase material

The thread-like materials used as raw material for the solid phase as well as their properties are listed in Table 1.

Example 6
The hCG-IEMA test

The following reagents were pipetted into test tubes provided with 1 cm pieces of thread coated with the antibody 5008:

1. 800 µl phosphate buffer containing NaCl, EDTA and BSA (Medix Biochemica Assay Buffer Cat 10803);
2. 200 µl standard: 0 (PBS), 50, 500, 5000, 50000 and 200000 IU/l hCG.

The mixture was incubated 30 minutes on a horizontal shaker at room temperature.

The tubes were washed three times with 3 ml of PBS containing 0.05 % Tween 20.

Thereafter, 100 µl of labelled antibody [antibody against hCG α-subunit (Medix Biochemica Clone Number 5503) conjugated to horseradish peroxidase (HRP) diluted in buffer solution (Assay Buffer Cat. No. 10803, Oy Medix Biochemica Ab, Finland)] was added and stirred for 30 minutes on a horizontal shaker at room temperature. The tubes were washed three times with 3 ml of PBS-0.05 % Tween 20 and 1000 µl of HRP substrate (ABTS) was added and stirred with a horizontal shaker at room temperature. The reaction was stopped with 500 µl of stopping solution (4 % oxalic acid) and the adsorbance at A414 nm was measured with a Multiscan photometer by transferring 300 µl of the mixture onto microtiter plates for the measurements.

Experiment 1

Threads No. 1-17 were coated with hCG 5008 antibody and tested using the hCG IEMA-test using standard solutions containing 0 and 5000 IU/l of hCG. The most useful materials for further development of a good hCG assay would be those with the highest possible deviation of absorbance at the concentrations measured (Abs_{5000}- Abs_0). The results are shown in Table 2, which indicates that the best results were obtainable with threads No. 1, 3, 9 and 17.

Table 1

Materials and properties of the threads tested

Sample No.	Trade Name	Producer	Material	Thickness mm	Properties, type, number of strands
1	Kotinaru	Piippo-Tuote Oy, Finland	polypropylene	≈0.6x2.5	multiple
2	Nailon siima	Vonka, Finland	nylon	0.43	one
3	Unvaxed floss	Butler GUM, U.S.A	not known	≈0.2	multiple unwaxed floss
4	Ultra floss	Oral-B, Ireland	not known	≈1-≈0.1	stretchable ultra floss
5	Sewing Thread	Sonal, Mez AG, Germany	100 % Polyamid transparent	0.14	one
6	Joustolanka	Inka, Finland	elastane fiber	0.04	stretchable
7	SILK,	Madeira, Germany	100% Pure silk	≈0.7	multiple
8	Silk, S 303	Gütermann, Germany	100% Silk	≈0.1	multiple
9	Bear (karhu)	J. & P. Coats, EEC	100% Polyester	≈0.15	multiple
10	Duet Cordonnet	Coats, EEC	100% polyester	≈0.13	multiple
11	Col.800	Gütermann, Germany	100% polyester	≈0.07	multiple
12	Stern-Stopfgarn	Coats Mez, Germany	75% wool 25% polyamid	≈0.3	multiple
13	Sewing thread	Mölnlycke, Sweden	Cotton	≈0.11	multiple
14	Perle Cotton	Anchor, Germany	100% cotton	≈0.15	multiple
15	Baumwolle	Coats, EEC	100% cotton	≈0.17	multiple
16	Transorb pre-filter	AFC Worldwide, U.S.A.	nylon fibers	0.76	bonded fibers
17	Lahjalanka	Not known, Finland	not known	5x0.12	bonded fibers
18	Transorb R-13038	AFC Worldwide, U.S.A.	bonded nylon	0.76	bonded nylon

Table 2

Sample No.	Abs. 414 nm 0 IU/l	Abs. 414 nm ≈5000 IU/l	Abs Difference	Thread cm/tube	Abs/cm
1	0.220	2.945	2.725	3	0,908
2	0.058	0.166	0.0108	7.35	0.015
3	0.084	1.280	1.196	9	0.133
4	0.066	0.132	0.066	12.5	0.005
5	0.059	0.655	0.596	36.5	0.016
6	0.068	0.113	0.045	27.5	0.002
7	0.114	0.172	0.058	9	0.006
8	0.071	0.166	0.095	29	0.003
9	0.166	1.966	1.830	15.5	0.118
10	0.184	1.073	0.889	14.25	0.062
11	0.072	0.590	0.518	27	0.019
12	0.058	0.146	0.088	14	0.006
13	0.070	0.398	0.328	25	0.013
14	0.325	0.660	0.328	12.5	0.027
15	0.136	0.600	0.464	18.25	0.025
16	0.241	0.484	0.243	10.75	0.023
17	0.107	2.177	2.070	13.5	0.153

Experiment 2

Threads No. 1, 3, 4, 5, 9 , 10, 11 and 17 from the previous experiment and a new type of thread No. 18 (Transorb R 13038) were chosen for further tests.

Because the threads may contain substances, which might disturb the adsorbtion of the protein, they were washed with two different protein removing detergent solutions, i.e. by ethanol and dioxine before coating.

Equal lengths of coated threads were added to tubes and measured with the hCG IEMA test at first using standards containing 0 to 5000 IU/hCG. The results are shown in Table 3 and 4 as well as in Figures 5 and 6, which indicate the results of ethanol- and dioxin-washed threads respectively. The best thread was No. 1, which showed the greatest deviation in the

absorbance.

Table 3
Threads washed with ethanol

Sample No.	Abs 414 nm/cm 0 IU/l	Abs 414 nm/cm ≈5000 IU/l	Abs/cm difference
1	0.013	3.236	3.123
3	-	-	-
4	0.060	0.664	0.604
5	0.057	0.063	0.006
9	0.077	0.634	0.557
10	0.109	0.429	0.320
11	0.055	0.221	0.166
17	0.074	1.270	1.196
18	0.087	0.214	0.127

Table 4
Threads washed with dioxine

Sample No.	Abs 414 nm/cm 0 IU/l	Abs 414 nm/cm ≈5000 IU/l	Abs/cm difference
1	0.091	3.022	2.931
3	0.151	0.279	0.128
4	0.060	0.421	0.361
5	0.047	0.070	0.023
9	0.057	0.645	0.588
10	0.063	0.629	0.566
11	0.056	0.207	0.151
17	0.061	0.711	0.650
18	0.054	0.342	0.288

Experiment 3

Experiment 3 was carried out in order to compare standard curves obtained by using different materials. The same threads were used as in the previous test, but several standard solutions, i.e. 0, 50, 500, 5000 and 50000 IU/l hCG). The results are shown in Tables 5-6.

Table 5
Threads washed with ethanol

Sample No.	Abs 414 nm/cm				
	0 IU/l hCG	50 IU/l hCG	500 IU/l hCG	5000 IU/l hCG	50000 IU/l hCG
1	0.068	0.131	0.728	2.754	2.918
3	ND	ND	ND	ND	ND
4	0.059	0.064	0.231	0.599	0.714
5	-	-	-	-	-
9	0.079	0.081	0.166	0.405	0.429
10	0.057	0.065	0.132	0.405	0.614
11	0.051	0.058	0.084	0.170	0.234
17	0.062	0.090	0.225	0.524	1.100
18	0.071	0.076	0.119	0.287	0.421

Table 6
Threads washed with dioxine

Sample No.	Abs 414 nm/cm				
	0 IU/l hCG	50 IU/l hCG	500 IU/l hCG	5000 IU/l hCG	50000 IU/l hCG
1	0.184	0.185	0.428	1.849	2.371
3	0.065	0.080	0.083	0.214	0.259
4	0.072	0.076	0.107	0.321	0.493
5	-	-	-	-	-
9	0.066	0.082	0.138	0.436	0.598
10	0.062	0.076	0.157	0.402	0.503
11	0.057	0.061	0.087	0.200	0.220
17	0.076	0.100	0.346	0.664	0.670
18	0.071	0.086	0.163	0.299	0.312

As a conclusion it was found that thread No. 3 was destroyed in ethanol-washing; thread No. 5 was discarded due to weak performance. Further it was concluded that no significant differences could be observed between threads washed with ethanol and dioxine, except for thread No. 1, which gave a high background absorbance with dioxine washing. Thread No. 17 possibly had the broadest measuring range, about 50-5000 IU/l, when washed with ethanol.

It was observed that the threads probably had been treated with substances, which inhibit protein binding and it is probable that ethanol and dioxine cannot totally remove these substances. It was concluded that the most sensitive test was obtainable with thread No. 1, which is a multistrand polypropylene thread.

In said test the best result was obtained with a thread of polypropene named Kotinaru manufactured by Piippo-Tuote Oy, Finland. As such, Kotinaru is somewhat too thick for automatic use, but a cheap and applicable surface can easily be developed from it.

WHAT IS CLAIMED IS:

1. A solid carrier for use in immunoassays comprising a flexible thread-like solid carrier coated with at least one specific binding substance.

2. The solid carrier of claim 1, wherein the thread-like solid carrier comprises a flexible, reelable, easily-running, single- or multiple-stranded material with a good tensile strength containing a coating area sufficient to bind a specific binding substance in such amounts that it allows detection of the desired analyte.

3. The solid carrier of claim 2, wherein the thread-like solid carrier is treated with a contaminant removing washing solution to improve the attachment of specific binding substances before coating with at least one specific binding substance.

4. The solid carrier of claim 1, wherein the thread-like solid carrier is precut in pieces of defined length for manual use.

5. The solid carrier of claim 1, wherein the thread-like solid carrier is spooled on a coil mountable on a support which support can be installed on an automatic or continuously working analytical device which transfers the thread-like solid carrier through the appropriate reagents and recording systems to another coil.

6. The method for performing immunoassays for at least one analyte, comprising the steps of:
(a) contacting a defined part of the thread-like solid carrier coated with a specific binding substance to allow the analyte of the sample to react with said specific binding substance for a time dependent of type and concentration of the analyte to be determined;

(b) bringing said thread-like solid carrier continuously or step-wise in contact with the reagents needed to give a detectable or measurable reactions and system for recording said reaction; and
(c) collecting the spent thread-like solid carrier on another coil.

7. A method of claim 6 for performing immunoassays for a multitude of different analytes wherein a separate coil comprising a thread-like solid carrier coated with a specific binding substance is inserted for each type of analyte to be determined.

8. A device comprising at least one coil for spooling an unused thread-like solid carrier coated with a specific binding substance which device can be mounted to a support which can be attached to a rotating arm of an automatic analysator.

9. The device of claim 8 mounted on a support which allows the thread-like solid carrier to be transferred through the samples, reagents and recording to another coil capable of collecting said thread-like solid carrier.

10. A set of devices or coils with thread-like solid carriers coated with different types of binding substances to be used in the method of claim 8.

11. An apparatus for continuous measurements comprising a
(a) a thread-like solid carrier coated with a specific binding substance;
(b) a coil for reeling said thread-like solid carrier of step (a);
(c) a support for the coil of step (b);
(d) a sample source;
(e) a device for immersing the thread-like solid carrier into or through said sample source of step (d)

(f) reagents required for providing a detectable or measurable reaction as well as system for recording the reaction; and

(k) a second mountable coil on which the thread-like solid carrier is reeled up after the reaction has been recorded.

12. The use of the thread-like solid carrier coated with a specific binding substrate in an apparatus for automated or continuously working immunoassays.

13. The use of the coil containing the thread-like solid carrier coated with a specific binding substance in an apparatus for automatized continuously working recording of the results of the immunoreaction.

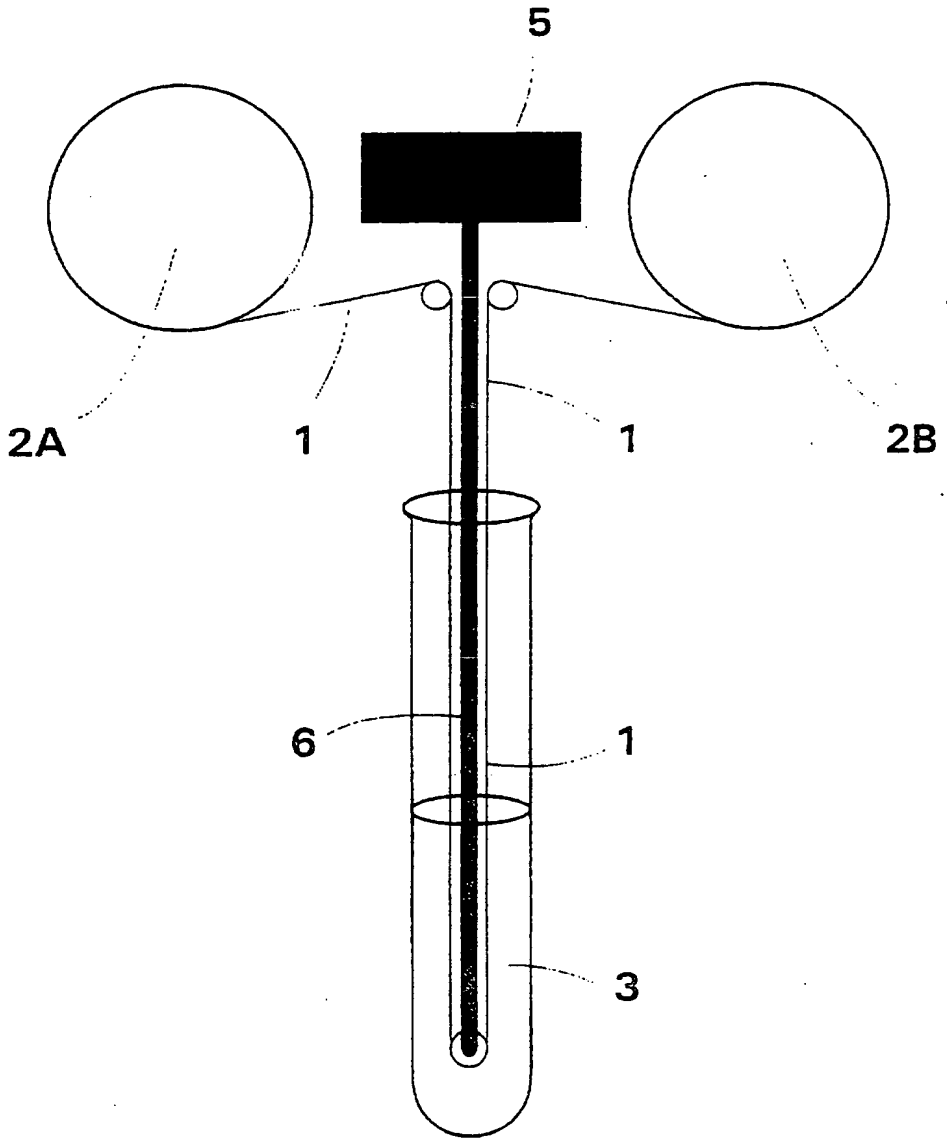


Fig. 1

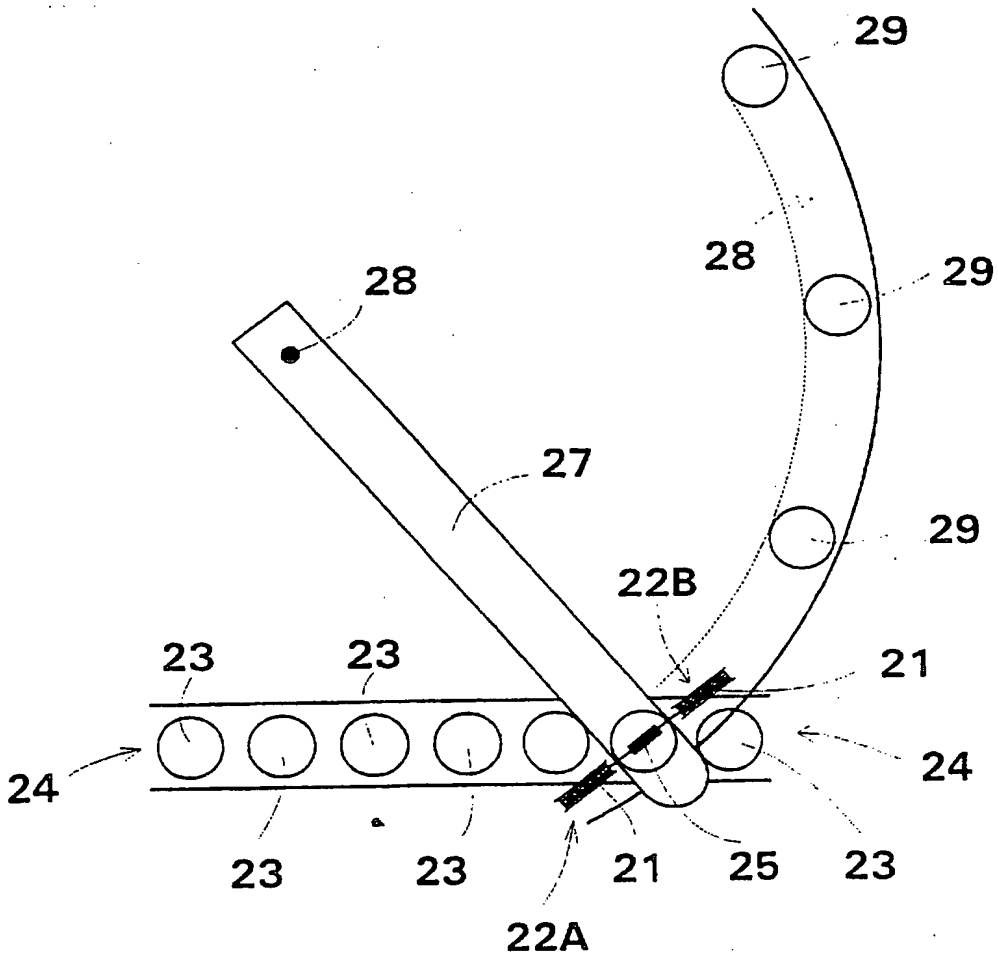


Fig. 2

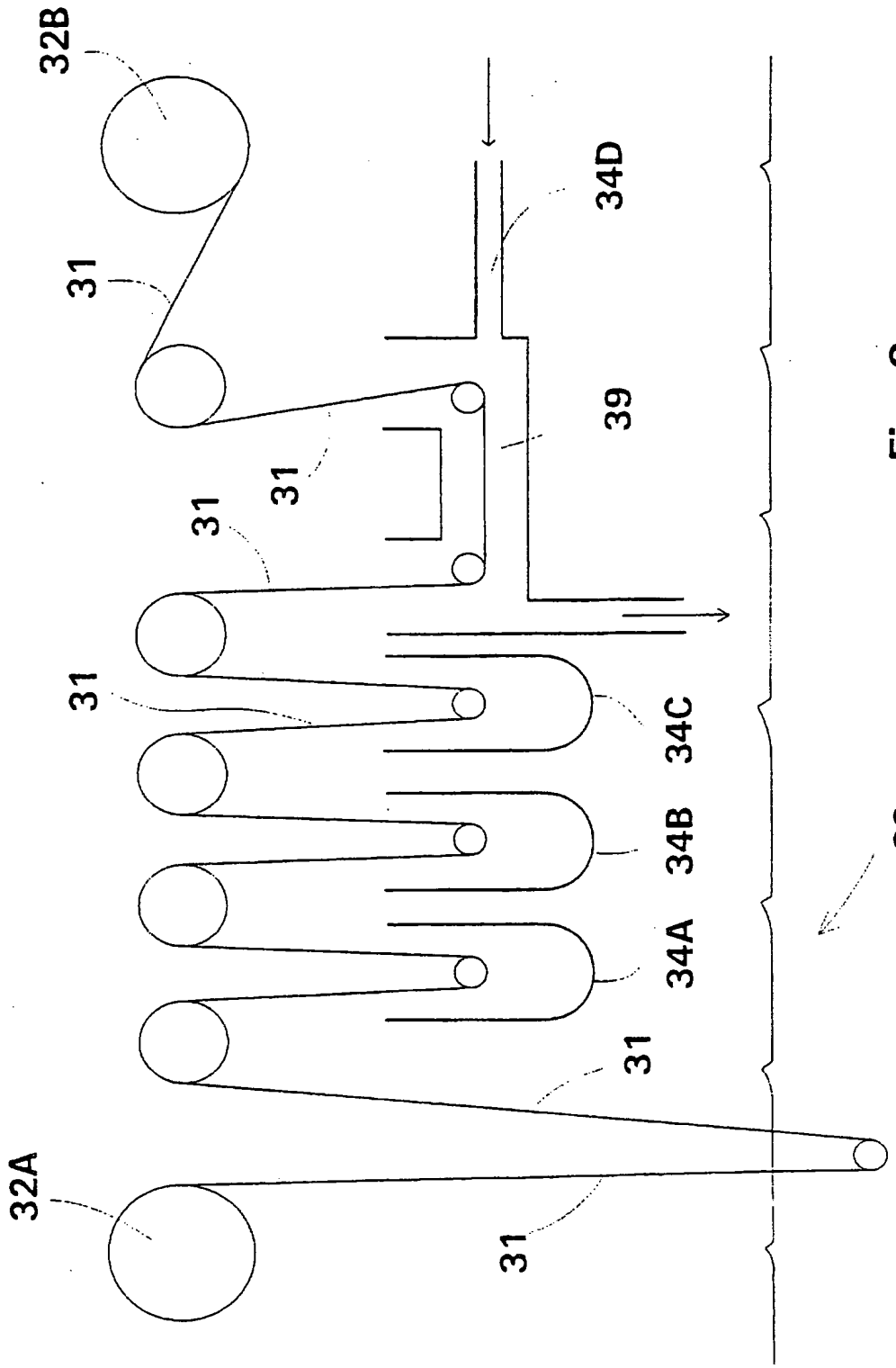


Fig. 3

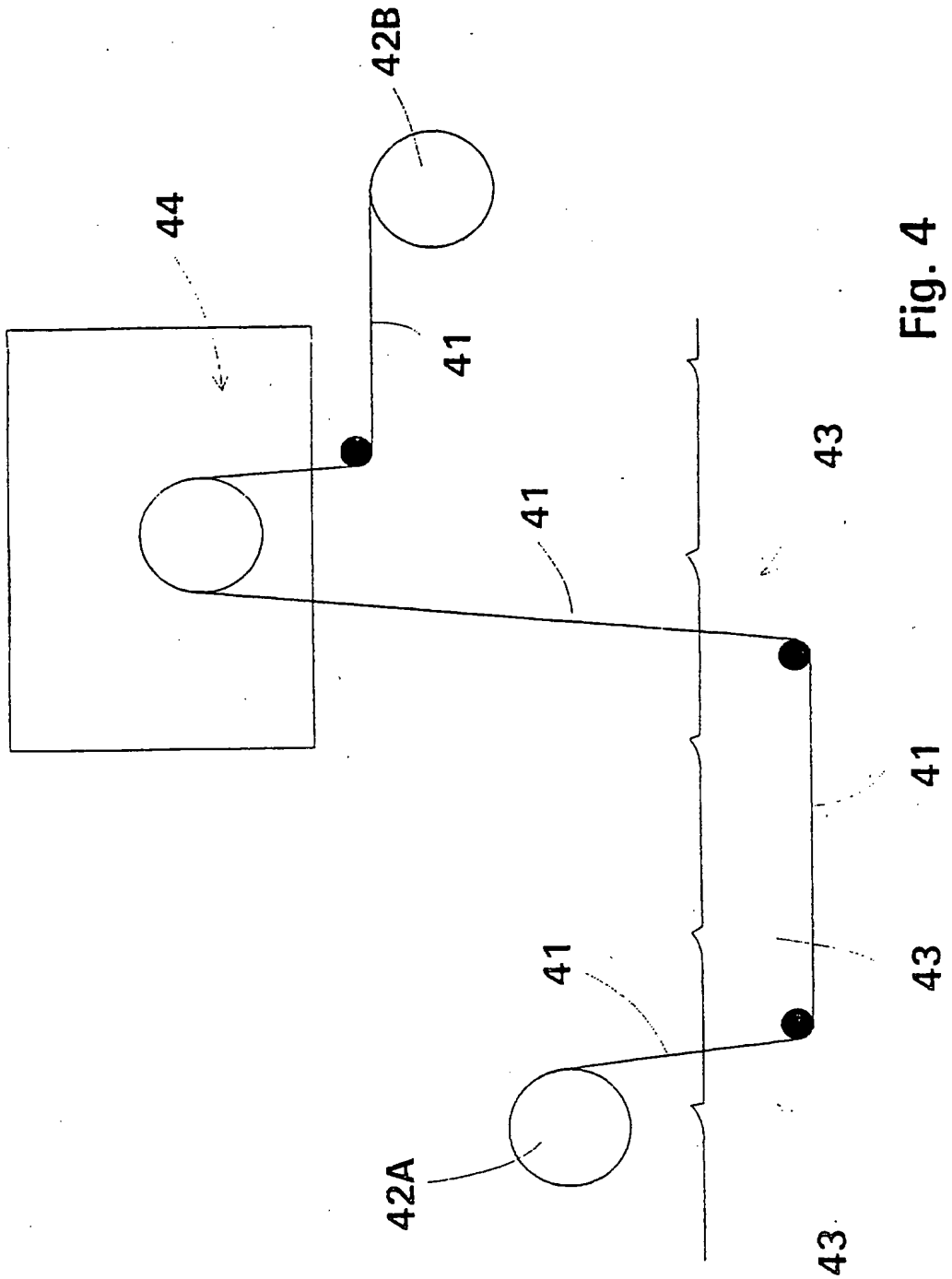


Fig. 4

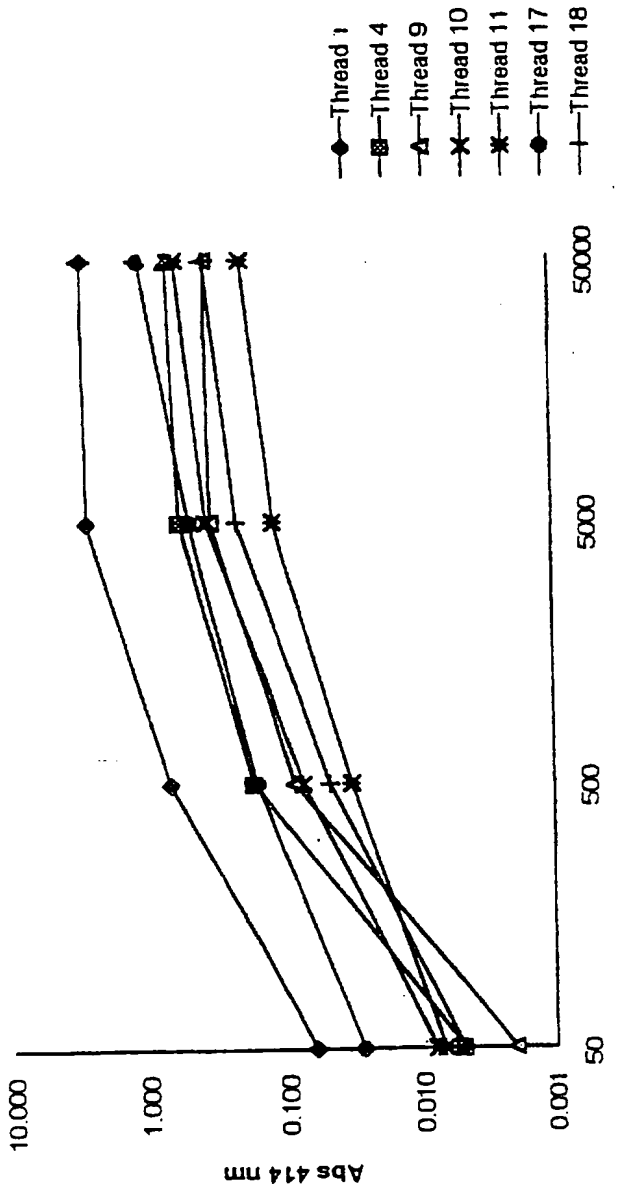
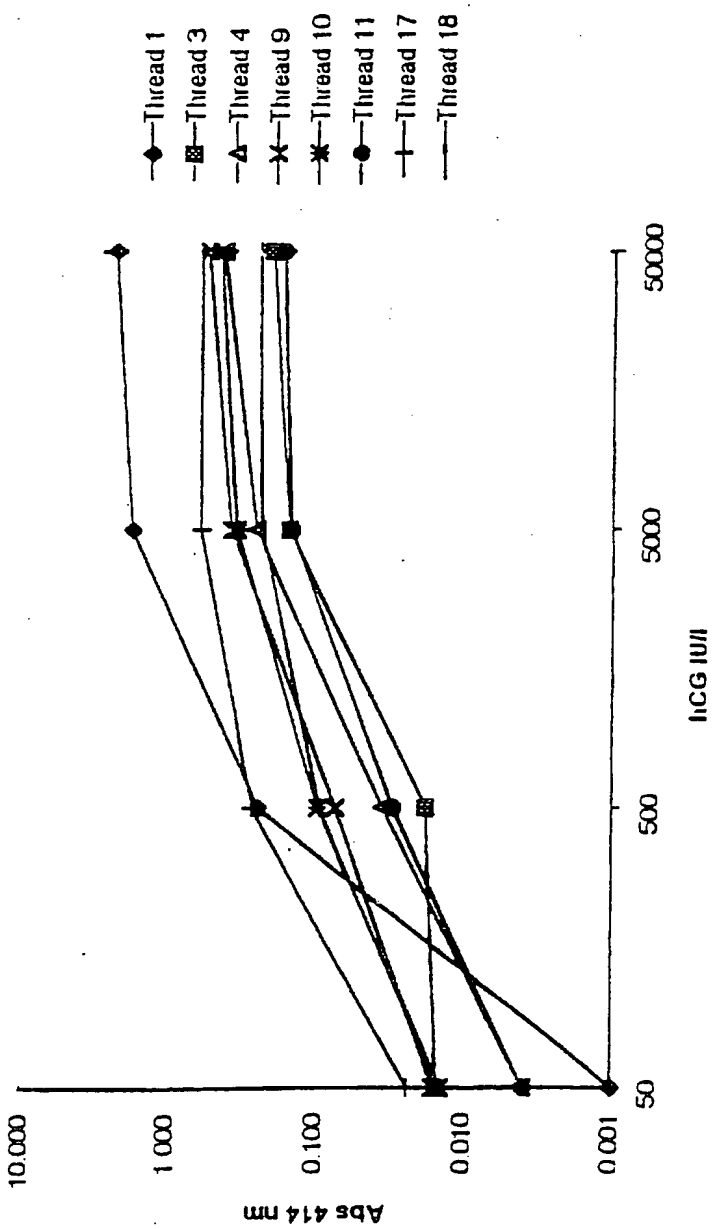

Thread 1
Thread 4
Thread 9
Thread 10
Thread 11
Thread 17
Thread 18
Abs 414 nm
10.000
1.000
0.100
0.010
0.001
50
500
5000
50000
hCG IU/l

Fig. 5


Thread 1
Thread 3
Thread 4
Thread 9
Thread 10
Thread 11
Thread 17
Thread 18
Abs 414 nm
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1.000
0.100
0.010
0.001
50
500
5000
50000
hCG IU/l

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 96/00442

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 33/543, G01N 35/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0139373 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 2 May 1985 (02.05.85)	1,2,4,5,8
Y		1-13
	--	
X	EP 0132285 A1 (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW), 30 January 1985 (30.01.85), see claims 11-13 and pages 2-4	1,2,4,5,8
Y		1-13
	--	
X	WO 8301308 A1 (MAST MEDICAL INDUSTRIES LTD.), 14 April 1983 (14.04.83), see claims	1
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 March 1997	29 -03- 1997
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 96/00442

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0119858 A2 (MAST IMMUNOSYSTEMS, LTD.), 26 Sept 1984 (26.09.84), see claims	1
Y	US 4071315 A (GUY CHATEAU), 31 January 1978 (31.01.78), see fig. 2 and columns 5-8	1-13
Y	WO 9311430 A1 (GEC-MARCONI LIMITED), 10 June 1993 (10.06.93), see claims and fig. 2	1-13
Y	File WPI, DERWENT accession no. 93-062348, Konica Corp: "Immunoassay device to detect trace amt. of substance in e.g. antigen antibody reaction - comprises lst tape shape member with e.g. antibodies fixed in water absorbing material, 2nd tape shape member of water absorbing material"; & JP,A,5010951, 930119, DW9308	1-13
A	File WPI, DERWENT asseccion no. 83-62101K, Unitika KK: "Carrier for immobilising physiologically active substances - comprises maleic anhydride -aromatic vinyl or olefin copolymer, maleic anhydride-aliphatic vinyl ether or ester copolymer, etc."; JP,A,58083633, 830519 DW8326	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

04/03/97

International application No.
PCT/FI 96/00442

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		JP-A- 60104260	08/06/85
		US-A- 4673657	16/06/87
EP-A1- 0132285	30/01/85	AU-A- 2985584	03/01/85
		JP-A- 60036958	26/02/85
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